Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

Listing of Claims

1. (Currently Amended) A replication competent polynucleotide comprising:

a 5' non-translated region (NTR), a 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, wherein the polyprotein comprises an isoleucine at about amino acid 2204, and further comprises ~~an~~ at least two adaptive mutations selected from the group of an arginine at about amino acid 1067, an arginine at about amino acid 1691, a valine at about amino acid 2080, an isoleucine at about amino acid 1655, an arginine at about amino acid 2040, an arginine at about amino acid 1188, and a combination thereof, wherein the replication competent polynucleotide is isolated, ~~and~~ wherein the amino acid sequence of the hepatitis C virus polyprotein and the amino acid sequence of SEQ ID NO:2 have at least about 90% identity, and wherein the 5' NTR, the 3' NTR, and the nucleotide sequence encoding the polyprotein are genotype 1a.

2. (Original) The replication competent polynucleotide of claim 1 further comprising a second coding sequence.

3. (Original) The replication competent polynucleotide of claim 2 wherein the second coding sequence encodes a marker.

4. (Original) The replication competent polynucleotide of claim 2 wherein the second coding sequence encodes a transactivator.

5. Canceled

6. (Original) The replication competent polynucleotide of claim 1 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

7. (Original) The replication competent polynucleotide of claim 1 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

8. (Original) The replication competent polynucleotide of claim 1 further comprising a nucleotide sequence having cis-acting ribozyme activity, wherein the nucleotide sequence is located 3' of the 3' NTR.

9-13. (Cancelled)

14. (Currently Amended) A method for making a replication competent polynucleotide comprising:

providing a polynucleotide comprising a 5' NTR, 3' NTR, a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, wherein the polyprotein comprises a serine at about amino acid 2204, a glutamine at about amino acid 1067, a lysine at about amino acid 1691, a phenylalanine at about amino acid 2080, a valine at about amino acid 1655, a lysine at about amino acid 2040, or a glycine at about amino acid 1188 and wherein the 5' NTR, the nucleotide sequence encoding the polyprotein, and 3' NTR are genotype 1a, wherein the amino acid sequence of the hepatitis C virus polyprotein and the amino acid sequence of SEQ ID NO:2 have at least about 90% identity; and

altering the coding sequence such that the polyprotein encoded thereby comprises an isoleucine at about amino acid 2204, and at least ~~one~~ two adaptive mutations selected from the group consisting of an arginine at about amino acid 1067, an arginine at about amino acid 1691, a valine at about amino acid 2080, an isoleucine at about amino acid 1655, an arginine at about amino acid 2040, an arginine at about amino acid 1188, and a combination thereof.

15. (Original) The method of claim 14 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

16. (Original) The method of claim 14 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

17. (Original) A replication competent polynucleotide produced by the method of claim 14.

18. (Currently Amended) A method for identifying a compound that inhibits replication of a replication competent polynucleotide, the method comprising:

contacting a cell comprising a replication competent polynucleotide with a compound, the replication competent polynucleotide comprising 5' NTR, 3' NTR, a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, wherein the hepatitis C virus polyprotein comprises an isoleucine at about amino acid 2204, and further comprises ~~an~~ at least two adaptive mutations selected from the group of an arginine at about amino acid 1067, an arginine at about amino acid 1691, a valine at about amino acid 2080, an isoleucine at about amino acid 1655, an arginine at about amino acid 2040, an arginine at about amino acid 1188, and a combination thereof, wherein the amino acid sequence of the hepatitis C virus polyprotein and the amino acid sequence of SEQ ID NO:2 have at least about 90% identity, and wherein the 5' NTR, the 3' NTR, and the nucleotide sequence encoding the polyprotein are genotype 1a;

incubating the cell under conditions wherein the replication competent polynucleotide replicates in the absence of the compound; and

detecting the replication competent polynucleotide, wherein a decrease of the replication competent HCV polynucleotide in the cell contacted with the compound compared to the replication competent polynucleotide in a cell not contacted with the compound indicates the compound inhibits replication of the replication competent polynucleotide.

19. (Original) The method of claim 18 wherein detecting the replication competent polynucleotide comprises nucleic acid amplification.

20. (Original) The method of claim 18 wherein the replication competent polynucleotide further comprises a second coding sequence encoding a marker, and wherein detecting the replication competent polynucleotide comprises identifying the marker.

21. (Original) The method of claim 18 wherein the replication competent polynucleotide further comprises a second coding sequence encoding a transactivator, wherein the cell comprises a polynucleotide comprising a transactivated coding sequence encoding a detectable marker and an operator sequence operably linked to the transactivated coding sequence, wherein the transactivator interacts with the operator sequence and alters expression of the transactivated coding sequence, and wherein detecting the replication competent polynucleotide in the cell comprises detecting the detectable marker encoded by the transactivated coding sequence.

22. (Original) The method of claim 18 wherein the cell is a human hepatoma cell.

23. (Original) The method of claim 18 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

24. (Original) The method of claim 18 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

25. Canceled

26. (Currently Amended) A method for selecting a replication competent polynucleotide, the method comprising:

incubating a cell in the presence of a selecting agent, wherein:

the cell comprises a polynucleotide comprising a 5' non-translated region (NTR), a 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, and a second coding sequence, wherein the polyprotein comprises an isoleucine at about amino acid 2204, and further comprises an at least two adaptive mutations selected from the group of an arginine at about amino acid 1067, an arginine at about amino acid 1691, a valine at about amino acid 2080, an isoleucine at about amino acid 1655, an arginine at about amino acid 2040, an arginine at about amino acid 1188, and a combination thereof, wherein the amino acid sequence of the hepatitis C virus polyprotein and the amino acid sequence of SEQ ID NO:2 have at least about 90% identity, and wherein the 5' NTR, the 3' NTR, and the nucleotide sequence encoding the polyprotein are genotype 1a;

the second coding sequence encodes a selectable marker conferring resistance to the selecting agent; and

the selecting agent inhibits replication of a cell that does not express the selectable marker; and

detecting a cell that replicates in the presence of the selecting agent, wherein the presence of such a cell indicates the polynucleotide is replication competent.

27. (Original) The method of claim 26 wherein the selecting agent is an antibiotic.

28. (Original) The method of claim 26 wherein the cell is a human hepatoma cell.

29. (Original) The method of claim 26 wherein the cell is a first cell, the method further comprising:

obtaining a virus particle produced by the first cell;

exposing a second cell to the isolated virus particle and incubating the second cell in the presence of the selecting agent; and

detecting a second cell that replicates in the presence of the selecting agent, wherein the presence of such a cell indicates the replication competent polynucleotide in the first

cell produces an infectious virus particle.

30. (Original) The method of claim 26 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

31. (Original) The method of claim 26 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

32. Canceled

33. (Currently Amended) A method for detecting a replication competent polynucleotide, the method comprising:

incubating a cell comprising a replication competent polynucleotide, wherein:

the replication competent polynucleotide comprises a 5' non-translated region (NTR), a 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, and a second coding sequence encoding a transactivator, wherein the polyprotein comprises an isoleucine at about amino acid 2204, and further comprises ~~an~~ at least two adaptive mutations selected from the group of an arginine at about amino acid 1067, an arginine at about amino acid 1691, a valine at about amino acid 2080, an isoleucine at about amino acid 1655, an arginine at about amino acid 2040, an arginine at about amino acid 1188, and a combination thereof, wherein the amino acid sequence of the hepatitis C virus polyprotein and the amino acid sequence of SEQ ID NO:2 have at least about 90% identity, and wherein the 5' NTR, the 3' NTR, and the nucleotide sequence encoding the polyprotein are genotype 1a;

the cell comprises a transactivated coding region and an operator sequence operably linked to the transactivated coding region; and

the transactivated coding region encodes a detectable marker, wherein the transactivator alters transcription of the transactivated coding region; and

detecting the detectable marker, wherein the presence of the detectable marker indicates the cell comprises a replication competent polynucleotide.

34. (Original) The method of claim 33 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

35. (Original) The method of claim 33 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

36. Canceled

37-40. (Cancelled)

41. (Original) A method for producing viral particles, comprising incubating a cell comprising the replication competent polynucleotide of claim 1 under conditions allowing the polynucleotide to replicate.

42. (Original) The method of claim 41 further comprising isolating the viral particles.

43. (Original) A method for using the viral particles of claim 41 in an assay.

44-46. (Cancelled)

47. (New) The replication competent polynucleotide of claim 1 wherein the replication competent polynucleotide replicates in Huh-7 cells.

48. (New) The method of claim 14 wherein the replication competent polynucleotide replicates in Huh-7 cells.

49. (New) The method of claim 22 wherein the cell is a Huh-7 cell.

50. (New) The method of claim 28 wherein the cell is a Huh-7 cell.

51. (New) The method of claim 33 wherein the cell is a human hepatoma cell.

52. (New) The method of claim 51 wherein the cell is a Huh-7 cell.